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A Population-based Case-Control Study of Urinary Arsenic Species and Squamous Cell Carcinoma in New Hampshire, USA

Diane Gilbert-Diamond[1], Zhigang Li[1], Ann E. Perry[2], Steven K. Spencer[3], A. Jay Gandolfi[4], and Margaret R. Karagas[1]

[1] Section of Biostatistics and Epidemiology, Department of Community and Family Medicine, Geisel School of Medicine at Dartmouth, Lebanon, New Hampshire, USA

[2] Department of Pathology, Geisel School of Medicine at Dartmouth, Lebanon, New Hampshire, USA

[3] Department of Surgery, Geisel School of Medicine at Dartmouth, Lebanon, New Hampshire, USA

[4] Department of Pharmacology & Toxicology, University of Arizona, Tucson, Arizona, USA

Corresponding author: Diane Gilbert-Diamond, Geisel School of Medicine at Dartmouth, Hinman Box 7937, One Medical Center Drive, Lebanon, NH 03756, 603 653 3362 (t), 603 653 9093 (fax), Diane.Gilbert-Diamond@Dartmouth.edu

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ABSTRACT

BACKGROUND: Chronic high arsenic exposure is associated with squamous cell carcinoma (SCC), and inorganic arsenic metabolites may play an important role in this association. However, little is known about the carcinogenicity of arsenic at levels commonly observed in the U.S.

OBJECTIVE: To estimate associations between total urinary arsenic and arsenic species and SCC in a U.S. population.

METHODS: We conducted a population-based case-control SCC study (n = 470 cases, 447 controls) in a U.S. region with moderate arsenic exposure through private well water and diet. We measured urinary inorganic arsenic (iAs), monomethylarsonic acid (MMA), dimethylarsinic acid (DMA), and summed these arsenic species (sAs). Participants who reported seafood consumption for 2 days before urine collection were excluded from analyses, as seafood contains arsenolipids and arsenosugars that metabolize into DMA through alternate pathways.

RESULTS: In adjusted logistic regression analyses (n = 323 cases, 319 controls), the SCC odds ratio (OR) was 1.37 for each unit increase in ln(sAs (μg/L)) (95% CI: 1.04, 1.80). Urinary ln(MMA) and ln(DMA) also were positively associated with SCC (OR = 1.34; 95% CI: 1.04, 1.71 and OR = 1.34; 95% CI: 1.03, 1.74, respectively). A similar trend was observed for ln(iAs) (OR = 1.20; 95% CI: 0.97, 1.49). Percent iAs, MMA, and DMA were not associated with SCC.

CONCLUSIONS: These results suggest that arsenic exposure at levels common in the U.S. relates to SCC and that arsenic metabolism ability does not modify the association.

INTRODUCTION

Nonmelanoma skin cancer is the most common type of malignancy among whites, and has considerable related morbidity and health costs (Mudigonda et al. 2010). The incidence rates of both forms of nonmelanoma skin cancer, cutaneous squamous cell carcinoma (SCC) and basal cell carcinoma (BCC), are dramatically increasing in the United States (Karagas et al. 1999; Rogers et al. 2010). While BCC is more common, SCC has a greater tendency to metastasize and is responsible for greater mortality (Weinstock et al. 1991). Sunlight exposure, male sex, and older age are well known risk factors for SCC (Preston and Stern 1992).

Chronic exposure to arsenic (As) has been associated with SCC in regions of Taiwan where high exposures through drinking water were common (Tseng et al. 1968; Yeh et al. 1968), however it is not clear whether lower levels of exposure that are common in the U.S. also are a risk factor for SCC. We previously reported increased odds of SCC in association with toenail As concentrations >97th percentile (OR = 2.07; 95% CI: 0.92, 4.66 compared to toenail As < median) in a case-control study in the U.S. state of New Hampshire (Karagas et al. 2001). Furthermore, we found evidence of a positive association with toenail As concentrations >0.105 µg/g (Karagas et al. 2002), but not with concentrations below that value. While toenail arsenic concentration is considered a reliable long-term biomarker of As exposure (Garland et al. 1993), toenails primarily accumulate inorganic As (iAs), so our previous analysis could not examine specific As species that result from As metabolism.

Upon ingestion, iAs is methylated into monomethylarsonic acid (MMA V) which is then reduced to monomethylarsonous acid (MMA III). In a second step, MMA III is methylated to

dimethylarsinic acic (DMA V), which is then reduced to dimethylarsinous acid (DMA III) (Vahter 2002). This process is incomplete, such that iAs, MMA V, and DMA V are all present in the urine, the primary route of arsenic excretion (Francesconi et al. 2002). MMA III and DMA III have been detected in the urine of highly arsenic-exposed people (Mandal et al. 2001; Valenzuela et al. 2005), but not in most studies of people exposed to lower levels of arsenic (Lindberg et al. 2006; Rivera-Núñez et al. 2011).

Arsenic methylation was initially considered a detoxification process because MMA V and DMA V are considered less toxic than iAs and are easily excreted (Gebel 2002; Moore et al. 1997). However, growing evidence suggests that MMA III and DMA III are more toxic than their pentavalent forms, and that they may even be more toxic than iAs (Mass et al. 2001; Petrick et al. 2000; Styblo et al. 2000). Given their differing toxicities, it is likely that various arsenic species have different health effects. Studies of populations exposed to high levels of arsenic suggest that the profile of urinary arsenic species may affect arsenic-related skin pathologies; for example, individuals who have lower concentrations of urinary MMA relative to DMA may be at lower risk of arsenic-induced skin lesions (Kile et al. 2011; Yu et al. 2000), non-melanoma skin cancers combined (Chen et al. 2003), and BCC (Leonardi et al. 2012). To our knowledge, no studies have specifically examined the association between arsenic metabolites and skin cancer in a U.S. population, nor have any studies focused specifically on SCC. We therefore extended our population-based case-control study in New Hampshire to investigate the relation between individual urinary As species and the incidence of SCC.

METHODS

In collaboration with over 90% of the dermatologists and pathologists practicing in New Hampshire and bordering areas, we identified incident cases of invasive SCC of the skin that were newly diagnosed in residents aged 25-74 years from July 2003 and June 2009. Study staff reviewed pathology logbooks and medical charts at participating dermatology and pathology clinics in order to identify all eligible SCC cases. We selected a control group of New Hampshire residents from The Center for Medicare and Medicaid services (for those 65 years and older) and driver's license records provided by the New Hampshire Department of Transportation (for those younger than 65 years), frequency matched to cases on sex and age (25-35, 36-45, 46-50, 51-59, 60-64, 65-69 and 70-74 years) (Applebaum et al. 2007). To be eligible, cases and controls were required to be English-speaking, mentally competent residents of New Hampshire, with a working telephone number. Cases and controls were still eligible to participate if they had a previous SCC that was diagnosed prior to the study period. The enrollment and interviews for cases and controls happened concurrently and at about the same rate over the course of the study. The current analysis includes cases and controls interviewed July 1 2006 to August 10 2011. Of 579 SCC cases confirmed eligible, we enlisted 510 (88%) and of the 594 controls, 483 participated (81%).

After study participants gave written informed consent, a study staff member who was blinded to their case/control status interviewed them in person to obtain sociodemographic, lifestyle, medical, and sun exposure information. Home tap water samples were collected from participants' homes in a commercially washed (mineral-free) high-density polyethylene bottle that meets EPA standards for water collection (I-Chem) by study staff wearing individually packed, clean, powderless gloves and following a strict protocol to limit contamination (Karagas

et al. 2001). Participants were mailed a urine-collection kit with instructions and materials necessary to collect a first-morning-void urine sample. The participants were provided with a prelabeled screw-top, 120 mL collection container that contained 30 μL of diammonium diethyldithiocarbamate to stabilize arsenic species. Collection materials also included an insulated thermos and instructions to refrigerate the urine sample in the thermos until giving it to the interviewer later that day. In addition, they were asked to complete a 3-day water, rice, and seafood intake record for the 3 days prior to urine collection. All procedures and study materials were approved by the Committee for the Protection of Human Subjects at Dartmouth College.

Water Arsenic Analysis

Tap water samples were tested using inductively coupled mass spectrometry (ICP-MS) at the Trace Element Analysis Core at Dartmouth using a quadrupole collision cell 7500c Octopole Reaction System ICP mass spectrometer (Agilent) and He as a collision gas to remove polyatomic interferences using previously described methods (Karagas et al. 2001). The detection limit of these analyses ranged from 0.002 to 0.075 μg/L, with detectable levels in over 96.7% of the samples. When water arsenic measurements were not available, the water arsenic concentration was set to half the value of the detection limit. The average coefficient of variation for these analyses was 3.22%.

Urinary Analysis

Urine samples from cases and controls were frozen at -80° C within 24 hours of collection and shipped on dry ice to University of Arizona where they were analyzed using a high-performance liquid chromatography (HPLC) ICP-MS system using previously described methods (Gilbert-Diamond et al. 2011). This arsenic speciation method quantifies the concentration of inorganic

arsenic species (iAs III and iAs V) and organic arsenic species (monomethylarsonic acid V (MMA), dimethylarsinic acid V (DMA), and arsenobetaine (AsB). No cases and controls were below the DMA limit of detection (0.11 μg/L); 3.6% of cases and 4.5% of controls were below the MMA limit of detection (0.14 μg/L); 32.3% of cases and 31.8% of controls were below the iAsIII limit of detection (0.15 μg/L); and 82.1% of cases and 87.5% of controls were below the iAsV limit of detection (0.10 μg/L). Urinary As values below the detection limit were set to half the value of the detection limit.

We calculated the sum of urinary inorganic and methylated arsenic species (sAs) by summing iAs, MMA and DMA. Because AsB is thought to pass through the body without being metabolized and is non-toxic, it was excluded from this analysis (Francesconi et al. 2002). We divided urinary iAs, MMA, and DMA by sAs in order to calculate the percentage of each arsenic species (%iAs, %MMA, %DMA). We calculated the ratio of urinary MMA to iAs as a biomarker of the primary methylation capability, and the ratio of urinary DMA to MMA as a biomarker of the secondary methylation capability (Chen et al. 2003). Urinary creatinine was measured with a colorimetric assay (Assay #500701, Caymen Chemical, Ann Arbor, MI).

Data Analysis

We first evaluated the association between individual urinary As species and factors that could potentially influence urinary arsenic concentrations in bivariate regression models among controls (n = 447). Urinary arsenic concentrations were natural log-transformed to improve normality. Self-reported rice consumption, including cooked rice and rice cereals, was converted into equivalents of cooked rice using previously described methods (Gilbert-Diamond et al. 2011). To focus on iAs and its methylation products, we restricted further analyses to

participants who did not report eating seafood in the prior two days. We chose a two-day window based on arsenic excretion dynamics (Buchet et al. 1981), though results were consistent in a sensitivity analysis that used a three-day window to define non-seafood consumers (data not shown). We fit a locally weighted smoothed trend through the unadjusted data to visually inspect the relationship between the proportion of SCC cases and urinary arsenic concentrations (S-PLUS 8.0; TIBCO Software, Inc.). We then fit individual generalized linear models with a logit transform to estimate odds ratios for SCC in association with a unit increase in urinary sAs, iAs, MMA, DMA, %iAs, %MMA, %DMA, iAs/MMA, and DMA/MMA. To improve normality of urinary arsenic species and reduce the influence of potential outliers, we also fit models with natural-log transformed urinary arsenic. We additionally estimated odds ratios for SCC according to tertiles of urinary As concentrations (based on the distribution in the controls) to explore possible non-linear associations. All models were fit with and without adjustment for sex, age (continuous), BMI (continuous), education (high school, college, graduate school), smoking status (at time of diagnosis/reference age: never, former, current), skin reaction to chronic sun exposure (very tan, moderately tan, mildly tan, freckle/no tan), and urinary creatinine concentration—a measure of urinary dilution (continuous). We only had resources to measure urinary creatinine in a subset of samples (n = 596) so multiple imputation using fully conditional specification methods was used to account for missing data (van Buuren 2007). Models for As percentages and ratios were additionally adjusted for water As concentration to control for potential confounding by iAs exposure. We performed a sensitivity analysis in which we additionally restricted analyses to non-rice eaters, because rice is known to contain DMA (Williams et al. 2005). In order to account for DMA introduced by unreported fish consumption, we also did a sensitivity analysis in which we further adjusted for urinary arsenobetaine. To

explore whether the association between urinary arsenic and SCC varied by duration of current water supply use, we stratified participants according to the median duration of current water supply use (<17 or ≥ 17 years) and estimated odds ratios for SCC using covariate-adjusted logistic regression models with ln-transformed urinary arsenic as the exposure. All regression analyses were performed with SAS version 9.2 (SAS Institute, Cary, North Carolina).

A structural equation model (SEM) was used to simultaneously model associations between SCC and urinary As metabolites (iAs, MMA, and DMA), water As concentration, and rice consumption. The three urinary As variables are highly correlated and thus ill-suited to be included in a single logistic regression model. The structural equation model permits iAs, MMA, and DMA to each load differently onto a single latent urinary arsenic variable (UAM) based on their correlation structure; the loading score of each arsenic species indicates its relative contribution to the variable. Robust weighted least square estimators were used to estimate parameters in the model. We used the comparative fit index (Bentler 1990) and Tucker Lewis index (Tucker and Lewis 1973) to evaluate the model fit. We calculated the indirect association between water arsenic and SCC and rice consumption and SCC, through the latent UAM variable, and tested its statistical significance using the Sobel Z test (Shrout and Bolger 2002). For the purpose of comparison, we also created a UAM model without the assumption of unobserved variables by replacing the latent UAM variable with the simple sum of the three urinary As metabolites in the model. The type I error rate used to define statistical significance is 0.05 for these models. The SEM analysis was conducted using Mplus version 6.12 (Muthén & Muthén, Los Angeles, California).

RESULTS

The average time between SCC diagnosis and interview/urine collection was 23 months (SD 10.2 months). Urine samples were collected on 470 SCC cases (92%) and 447 controls (75%), resulting in a total sample size of 917. The sex and age distributions and percentage of urban versus rural did not differ for eligible cases and those who participated in our study. The sex distribution and percentage of urban versus rural also did not differ between identified controls and those who participated in our study; however, controls who did participate tended to be older (63 years vs. 56 years). In cases interviewed for our study, 98.5% of those ages 25 to 64 years had a valid driver's license at the time of interview, and 98% of those ages 65-74 were enrolled in Medicare, thus indicating that most would have been included in the data sources used to identify controls. The sex and age group distributions for SCC cases and controls were similar and the vast majority of all participants reported their race as white (Table 1). In addition, urban versus rural residence and the type of household water supply and length of use were similar between cases and controls, with 48% of all participants consuming water from a shared or private well and an overall median (25%, 75%) water supply usage of 17 years (8, 33 years). The consumption of fish and rice in the two days prior to urine collection was also similar in cases and controls. Compared to controls, cases generally had an increased sun sensitivity measured as a lower tendency to tan with chronic sun exposure (Table 1). On average, cases also had a lower body mass index, higher level of education and lower likelihood to have ever smoked when compared to controls.

Median urinary sAs, iAs, MMA, and DMA concentrations were higher in cases than controls (Table 1). In bivariate analyses restricted to controls, age, BMI, and water arsenic concentration were associated with natural log transformed concentrations of urinary sAs, iAs, MMA, and

DMA (Supplemental Material, Table S1). Additionally, being male was associated with higher urinary Ln(iAs) and Ln(MMA) and having a higher education level was associated with higher urinary Ln(sAs), Ln(iAs) and Ln(DMA). Seafood consumption was associated with higher urinary sAs, largely due to higher urinary DMA; each 4 oz. serving of seafood was associated with an estimated 53% (95% CI: 37%, 71%) increase in urinary DMA concentration.

Among participants who reported not eating seafood in the two days prior to urine collection (323 cases, 319 controls), we observed a linear association between SCC and sAs, MMA, and DMA (Table 2). After adjustment for sex, age (continuous), BMI (continuous), education (high school, college, graduate school), smoking status (at time of diagnosis/reference age: never, former, current), skin reaction to chronic sun exposure (very tan, moderately tan, mildly tan, freckle/no tan) and urinary creatinine (continuous), the odds ratio of SCC was 1.33 for each μg/L increase in urinary MMA (95% CI: 1.04, 1.70) (Table 2). Urinary sAs and DMA were also associated with increased odds of SCC. Positive associations with SCC were also observed after ln-transforming the urinary arsenic variables. The OR for each ln(μg/L) increase of ln(sAs) was 1.37, 95% CI: 1.04, 1.80. The OR for each ln(μg/L) increase in ln(MMA) and ln(DMA) were 1.34, 95% CI: 1.04, 1.71 and 1.34, 95% CI: 1.03, 1.74, respectively. In contrast, SCC was not associated with the percentage of urinary arsenic species or arsenic methylation ratios (Supplemental Material, Table S2). Results were not sensitive to creatinine adjustment (data not shown). Results were also not substantially different when using a three-day window to define seafood consumers, nor when further adjusting for urinary AsB (data not shown). Regression coefficients were also similar in unadjusted and adjusted models after excluding an additional 65 cases and 59 controls who reported eating rice in the previous two days, though the smaller sample size resulted in slightly wider confidence intervals (Supplemental Material, Table S3).

Urinary MMA concentration was also significantly associated with odds of SCC when urinary As was examined by tertiles of exposure (Table 2). Participants in the third tertile of urinary MMA had a 76% higher odds of SCC compared to those in the first tertile, (OR = 1.76, 95% CI: 1.09, 2.84). Participants in the third tertiles of urinary sAs, iAs, and DMA also had increased odds of SCC, though, none of the odds ratios reached statistical significance.

In an analysis stratified by the median duration of current water supply usage, odds ratios for SCC were positive in association with unit increases in ln-transformed urinary As species in both strata (<17 years and ≥17 years) (Table 3). Odds ratios were somewhat larger among those with a shorter duration of current water usage, but the differences in the slopes between the two strata were not statistically significant.

In an SEM adjusted for the same set of covariates, iAs, MMA, and DMA each contributed significantly to the latent UAM variable (loading scores = 0.80, 0.52, 0.49, respectively) (Supplemental Material, Figure S1). The Comparative Fit Index (>0.90) and Tucker Lewis Index (>0.90) indicated a reasonable model fit. We found an association between the latent UAM variable and SCC (OR per SD increase = 2.04, 95% CI: 1.35, 3.10). Water arsenic concentration was associated with the latent UAM variable (SD increase in UAM for each 10 μg/L increase in water As = 0.32, 95% CI: 0.30, 0.34) and the indirect association between water arsenic concentration and SCC through UAM was also significant (OR = 1.02 for every μg/L increase in water As, 95% CI: 1.01, 1.04). The structural equation model that did not assume unobserved variables (Supplemental Material, Figure S2) also showed a significant, but weaker association between sAs and SCC (OR for each 1 unit increase of sAs was 1.02, 95% CI: 1.00, 1.03).

DISCUSSION

In this US population-based case-control study of participants living in a region with detectable arsenic in drinking water, we estimated positive associations between SCC and urinary sAs, MMA, and DMA concentrations. The results of our SEM analysis also suggest that a latent urinary arsenic variable representing urinary iAs, MMA, and DMA is associated with SCC. In contrast, percentages and ratios of the individual metabolites were not associated with SCC in the study population.

In our analysis, we were interested in ingested iAs and the products of its methylation, MMA and DMA. In our population, where water arsenic concentrations are relatively low, dietary sources of iAs can play a more substantial role in overall arsenic exposure (European Food Safety 2009). For example, previous studies have found rice to be a significant predictor of urinary iAs (Cascio et al. 2011; Gilbert-Diamond et al. 2011). In our adult study population, however, the prevalence of rice consumption was only 21% in the 2 days prior to urine collection and was not significantly associated with urinary iAs (Supplemental Material, Table S1). Seafood is the main dietary source of organic arsenic compounds including arsenobetaine, arsenolipids and arsenosugars, and the latter two compounds are metabolized into DMA (Cullen and Reimer 1989; Francesconi 2010). Other studies have reported seafood consumption as a major predictor of urinary DMA in U.S. populations (Navas-Acien et al. 2011; Rivera-Núñez et al. 2012) as we observed in our study population (Supplemental Material, Table S1). Given the different biotransformation pathways of iAS and organic arsenic compounds that result in DMA, and their potential differing toxicity (Cullen and Reimer 1989), we excluded participants who reported seafood consumption for the two days prior to urine collection. Though rice consumption was a significant predictor of urinary DMA in our study (Supplemental Material, Table S1), we chose

not to exclude rice consumers from our analysis, because we were interested in all potential sources of ingested iAs. However, results of sensitivity analyses that excluded rice consumers were generally consistent with the main analyses (Supplemental Material, Table S3).

Previous ecological studies in the arsenic endemic region of southwest Taiwan (Tseng et al. 1968; Yeh et al. 1968) support an association between high levels of exposure to arsenic through well water and SCC. The results from our study of urinary arsenic, consistent with our previous work using toenail arsenic as a biomarker, suggest that arsenic's association with SCC extends to lower levels of As exposure. To our knowledge, there are no other studies of urinary arsenic species and SCC in populations with similar levels of arsenic exposure for direct comparison. There are studies in more highly exposed populations that examined urinary arsenic species and arsenic-induced skin pathologies. The Health Effects of Arsenic Study (HEALS) in Bangladesh, reported that total urinary arsenic was associated with the risk of incident skin lesions (including melanosis and keratosis, known precursors to skin cancer (Sober and Burstein 1995; Woolgar and Triantafyllou 2011)), in a cohort of 10,182 adults (Argos et al. 2011). In a cross-sectional study of 76 residents of an arsenic endemic region of Mexico exposed to drinking water of ≥50 μg As/L, urinary MMA III concentrations were significantly higher among those with arsenic-related skin lesions (n = 55), whereas MMA V, DMA III or DMA V, iAs III or iAsV, and the sum of inorganic and methylated arsenic species were not significantly different from concentrations in residents without skin lesions (n = 21) (Valenzuela et al. 2005). The percentage or ratio of urinary metabolites of arsenic is thought to reflect individual differences in the ability to metabolize iAs, and there is some evidence that they reflect increased susceptibility to arsenic-induced skin lesions. In southwest Taiwan, a case-control study (n = 76 cases, 224 controls) reported that a low ratio of urinary DMA to MMA was positively associated with skin cancer

(BCC or SCC) in participants with high lifetime exposure to arsenic (>15mg/L-year) (Chen et al. 2003). In another, smaller Taiwanese case control study (n = 26 cases, 26 controls), those with >15.5% MMA had a significantly higher odds of skin disorders (including BCC, SCC, and hyperkeratosis/hyperpigmentation) compared to those with ≤15.5% MMA (OR = 5.5) (Yu et al. 2000). In the HEALS study, urinary %MMA and the ratio of DMA to MMA were significantly associated with skin lesions (including keratosis and melanosis) (Ahsan et al. 2007) in 594 cases and 1,041 controls; the top quartile of %MMA (median: 20.2%) was associated with an increased odds of skin lesions compared to the lowest quartile (median 7.5%) (OR = 1.57, 95% CI: 1.10, 2.26), and the top quartile of DMA/MMA (median: 10.9) was associated with a reduced odds of skin lesions compared to the lowest quartile (median 3.1) (OR = 0.64, 95% CI: 0.44, 0.91). Another large Bangladeshi case-control study of skin lesions (including SCC as well as keratosis and melanosis) (n = 859 cases, 868 controls) reported an odds ratio of 1.56, 95% CI: 1.15, 2.12, associated with each increase in $\log_{10}$ percentage of MMA (Kile et al. 2011). At the relatively low levels of As exposure in our population, SCC was associated with the absolute concentrations of MMA and DMA measured in urine, but not the relative proportions of MMA, DMA and iAs. Thus, it is not clear if differences in the proportion or ratio of arsenic metabolites reflect susceptibility to skin cancers at lower levels of exposure.

The observed association between methylated forms of As and SCC may reflect the carcinogenicity of those methylated forms or their chemical predecessors. Exposure to inorganic As is known to be carcinogenic, though the biological mechanisms are not yet fully understood (International Agency for Research on Cancer (IARC) 2004). In experimental systems, iAs exposure leads to increased generation of reactive oxygen species (ROS) which in turn lead to DNA damage as well as increased oxidative sensitive gene expression (Shi et al. 2004). For

example, a recent *in vitro* study in human immortalized lung epithelial cells and adenocarcinoma cells reported that arsenic-induced ROS generation activated protein kinase AKT and the extracellular signal-regulated kinase (ERK1/2) leading to increased downstream expression of endothelial growth factor (VEGF), an important angiogenesis regulator (Liu et al. 2011). Inorganic As may also be carcinogenic through promoting mitochondrial activity, a process that could fuel the increased energy demands of quickly replicating cells as well as lead to increased ROS generation (Lee et al. 2011). Arsenic exposure may also promote carcinogenesis through altering the methylation of oncogenes and tumor suppressor genes (reviewed by (Cheng et al. 2012)). This altered DNA methylation may result from a depletion of cellular *S*-adenosylmethionine (SAM) (Coppin et al. 2008; Reichard et al. 2007; Zhao et al. 1997), an essential methyl donor, by the arsenic methylation pathway. It is still unclear whether iAs methylation products are equally, or even more, carcinogenic compared to iAs. An *in vitro* study of human epidermal keratinocytes showed that trivalent and pentavalent MMA and DMA affected cellular viability, proliferation, and cytokine excretion to approximately the same extent as their inorganic counterparts (Vega et al. 2001). Another study of human keratinocytes showed evidence that MMA III may be even more cytotoxic than iAs III (Styblo et al. 2000). There is also *in vitro* evidence of greater genotoxicity of MMA III (Mass et al. 2001; Schwerdtle et al. 2003) and DMA III (Schwerdtle et al. 2003) compared to iAs III and that the genotoxicity is mediated by ROS (Nesnow et al. 2002). Further work is necessary to clarify the biological mechanisms of arsenic's carcinogenicity and to understand the relative carcinogenicity of iAs and its metabolites in the *in vivo* context.

In our case-control study, we were unable to measure urinary arsenic prior to SCC diagnosis. We therefore cannot exclude the possibility that cases could have changed some behaviors related to

arsenic exposure after their diagnosis and prior to urine collection. In addition, it is possible that SCC treatment could have resulted in changes in arsenic excretion; however, this is unlikely as the primary treatment for SCC is surgical removal of the carcinoma. Prospective studies are needed to better understand the temporality of the observed association. Prospective studies can also elucidate the latency period of arsenic exposure and SCC as the latency period of low-dose arsenic's relation with SCC is unknown.

Another limitation of our study is that we have a single urinary arsenic measurement, which may not adequately reflect long-term exposure. In a US study by Navas-Acien (n = 60) of Native American populations found that urinary arsenic excretion (including urinary As concentrations and percent of urinary As species) remained fairly constant over a 10 year period (Navas-Acien et al. 2009). A study in Bangladesh by Kile et al. that measured urinary arsenic in 196 participants over 2 years also found that the concentration of urinary As species were fairly reproducible over time (Kile et al. 2009), but that the percent As species varied. Studies from Chile (n = 73) (Hopenhayn-Rich et al. 1996) and the US (n = 81)(Steinmaus et al. 2005) reported that proportions of As species were fairly stable over time, but only over 2 months and 1 year, respectively. Overall, findings from previous studies suggest that a single urinary measurement may be a reliable measurement of exposure for a period of months to years. We recognize, however, that the latency period for arsenic exposure could be very long. Our participants tended to use the same water source for many prior years (median = 17 years) and while water arsenic concentration was associated with urinary arsenic (Supplemental Material, Table S1), other factors such as diet also influence arsenic exposure (European Food Safety 2009). Odds ratios stratified by the duration of current water system use did not significantly differ (Table 3). Our study was also limited by the fact that we were unable to measure arsenic concentrations directly

in the tissue of interest, the skin. Further understanding about arsenic metabolism, tissue distribution, and excretion is necessary to understand the biological interpretation of the associations between urinary arsenic and SCC in our study population.

In conclusion, using sensitive methods to measure urinary As exposure in a population-based study of SCC in residents of New Hampshire, urinary concentrations of sAs, MMA, and DMA were significantly associated with SCC. These findings suggest that common exposure levels may influence cancer risk in the US and elsewhere.

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Table 1. Selected characteristics of squamous cell carcinoma cases and controls

Variable	Squamous cell carcinoma (n = 470) n (%) or median (IQR)	Controls (n = 447) n (%) or median (IQR)
Sex		
Men	284 (60.4)	258 (57.7)
Women	186 (39.6)	189 (42.3)
Age (y)		
<50	19 (4.0)	34 (7.6)
50-59	91 (19.4)	83 (18.6)
60-69	213 (45.3)	203 (45.4)
≥ 70	147 (31.3)	127 (28.4)
Body mass index (kg/m^2)		
18.5-24.9	155 (33.4)	127 (28.6)
25.0-29.9	178 (38.4)	161 (36.3)
>30.0	125 (26.9)	153 (34.5)
Missing (n)	6	3
Race		
White	465 (99.8)	439 (98.7)
Non-white	1 (0.2)	6 (1.3)
Missing (n)	4	2
Skin reaction to chronic		
Very tan	69 (14.7)	134 (30.0)
Moderately tan	239 (51.1)	235 (52.7)
Mildly tan	121 (25.9)	60 (13.5)
Freckle/no tan	39 (8.3)	17 (3.8)
Missing (n)	2	1
Smoking[a]		
Never smoker	207 (44.0)	160 (35.8)
Former smoker	207 (44.0)	212 (47.4)
Current smoker	56 (11.9)	75 (16.8)
Highest level of		
High school	108 (24.7)	181 (40.5)
College	186 (40.9)	160 (35.8)
Graduate School	153 (34.3)	106 (23.7)
Missing (n)	1	0
Residence		
Urban	64 (13.6)	48 (10.7)
Rural	406 (86.4)	399 (89.3)
Household water supply		
Public	188 (40.8)	203 (46.4)
Shared well	27 (5.9)	20 (4.6)
Private well or spring	242 (52.5)	214 (48.9)
Missing (n)	9	9

Variable	Squamous cell carcinoma (n = 470) n (%) or median (IQR)	Controls (n = 447) n (%) or median (IQR)
Seafood Consumption		
Yes	128 (28.6)	147 (31.3)
No	319 (71.4)	323 (68.7)
Missing (n)	22	18
Rice Consumption		
Yes	96 (21.5)	98 (20.9)
No	351 (78.5)	372 (79.2)
Missing (n)	23	21
Years used water supply	17 (8, 32)	18 (8, 34)
Water arsenic (μg/L)	0.33 (0.14, 1.11)	0.31 (0.12, 0.94)
Urinary arsenic (μg/L)		
sAs[b]	5.27 (3.38, 8.52)	4.76 (2.94, 8.10)
iAs	0.34 (0.13, 0.59)	0.30 (0.13, 0.54)
MMA	0.52 (0.31, 0.82)	0.45 (0.29, 0.75)
DMA	4.37 (2.72, 7.25)	3.73 (2.37, 6.70)
AsB	7.36 (1.69, 29.01)	5.79 (1.13, 26.34)

iAs: inorganic arsenic

MMA: monomethylarsonic acid

DMA: dimethylarsinic acid

AsB: Asenobetaine

[a]Assessed at time of diagnosis/reference age

[b]Sum arsenic (sAs) is the sum of inorganic arsenic (iAs), monomethylarsonic acid (MMA), and dimethylarsinic acid (DMA)

Table 2. Unadjusted and adjusted odds ratios (95% confidence intervals) of squamous cell carcinoma by urinary arsenic concentration units or tertiles among cases and controls who reported no seafood consumption for the 2 days prior to urine sample (323 cases, 319 controls)

Predictor	Unadjusted[a]	Adjusted[b]
Untransformed urinary As		
sAs[c] (μg/L)	1.03 (1.00, 1.05)	1.03 (1.00, 1.06)
iAs (μg/L)	1.21 (0.96, 1.53)	1.26 (0.98, 1.61)
MMA (μg/L)	1.30 (1.03, 1.64)	1.33 (1.04, 1.70)
DMA (μg/L)	1.03 (1.00, 1.06)	1.03 (1.00, 1.07)
Natural log transformed urinary As		
ln(sAs[c] (μg/L))	1.29 (1.04, 1.60)	1.37 (1.04, 1.80)
ln(iAs (μg/L))	1.19 (0.99, 1.43)	1.20 (0.97, 1.49)
ln(MMA (μg/L))	1.26 (1.04, 1.53)	1.34 (1.04, 1.71)
ln(DMA (μg/L))	1.27 (1.03, 1.56)	1.34 (1.03, 1.74)
Urinary As tertiles[d]		
sAs		
tertile 1 (<3.36 μg/L)	1 (ref)	1 (ref)
tertile 2 (3.36 to <5.31 μg/L)	0.93 (0.63, 1.38)	0.94 (0.60, 1.45)
tertile 3 (≥5.31 μg/L)	1.39 (0.96, 2.03)	1.43 (0.91, 2.27)
iAs		
tertile 1 (<0.23 μg/L)	1 (ref)	1 (ref)
tertile 2 (0.23 to <0.45 μg/L)	0.92 (0.63, 1.36)	0.97 (0.63, 1.48)
tertile 3 (≥0.45 μg/L)	1.30 (0.89, 1.90)	1.27 (0.82, 1.98)
MMA		
tertile 1 (<0.35 μg/L)	1 (ref)	1 (ref)
tertile 2 (0.35 to <0.59 μg/L)	1.01 (0.68, 1.51)	1.06 (0.68, 1.65)
tertile 3 (≥0.59 μg/L)	1.61 (1.10, 2.35)	1.76 (1.09, 2.84)
DMA		
tertile 1 (<2.61 μg/L)	1 (ref)	1 (ref)
tertile 2 (2.61 to <4.18 μg/L)	0.93 (0.63, 1.38)	0.92 (0.60, 1.43)
tertile 3 (≥4.18 μg/L)	1.47 (1.01, 2.13)	1.53 (0.97, 2.41)

[a]From general linear models with the logistic link function and squamous cell carcinoma case/control status as the outcome. Values are the odds ratios for models with untransformed urinary As, natural log transformed urinary As, and tertiles as predictors.

[b]Models are additionally adjusted for sex, age (continuous), BMI (continuous), education (high school, college, graduate school), smoking status at diagnosis/reference age (never, former, current), and skin reaction to chronic sun exposure (very tan, moderately tan, mildly tan, freckle/no tan), and urinary creatinine concentration (continuous).

[c]Sum arsenic (sAs) is the sum of inorganic arsenic (iAs), monomethylarsonic acid (MMA), and dimethylarsinic Acid (DMA); arsenobetaine was not included in this total.

[d]Tertiles were calculated using the urinary arsenic distribution of the study controls.

Table 3. Adjusted odds ratios (95% confidence intervals) of squamous cell carcinoma by natural log transformed urinary arsenic concentration among participants who reported no seafood consumption for the 2 days prior to urine sample (323 cases, 319 controls). Data is stratified at the sample median of current water supply use duration.

	Years using current water supply[a]		*P* value for difference in odds ratios[b]
Predictor	<17 years (n = 163 cases, 159 controls)	≥17 years (n = 160 cases, 160 controls)	
ln(sAs[c] (μg/L))	1.55 (1.04, 2.32)	1.20 (0.81, 1.80)	0.38
ln(iAs (μg/L))	1.29 (0.95, 1.76)	1.09 (0.79, 1.50)	0.51
ln(MMA (μg/L))	1.59 (1.10, 2.32)	1.14 (0.80, 1.64)	0.21
ln(DMA (μg/L))	1.46 (0.99, 2.14)	1.22 (0.83, 1.79)	0.45

[a]From general linear models with the logistic link function, natural log transformed urinary As as the predictor and squamous cell carcinoma case/control status as the outcome. Models are adjusted for sex, age (continuous), BMI (continuous), education (high school, college, graduate school), smoking status (never, former, current), and skin reaction to chronic sun exposure (very tan, moderately tan, mildly tan, freckle/no tan), and urinary creatinine concentration.

[b]*P* value for a *t* test on the difference of the slopes, assuming unequal variances.

[c]Sum arsenic (sAs) is the sum of inorganic arsenic (iAs), monomethylarsonic acid (MMA), and dimethylarsinic Acid (DMA); arsenobetaine was not included in this total.